2020

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10.1016/j.bbagen.2020.129736

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Resistance mechanisms to targeted therapy in BRAF-mutant melanoma - A mini review

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ARTICLE INFO

Keywords:  
Intrinsic resistance  
Tumour adaptations  
Acquired resistance  
Targeted therapy  
Tumour plasticity  
Metabolic reprogramming

ABSTRACT

Background: The introduction of targeted therapies for the treatment of BRAF-mutant melanomas have improved survival rates in a significant proportion of patients. Nonetheless, the emergence of resistance to treatment remains inevitable in most patients.

Scope of review: Here, we review known and emerging molecular mechanisms that underlay the development of resistance to MAPK inhibition in melanoma cells and the potential strategies to overcome these mechanisms.

Major conclusions: Multiple genetic and non-genetic mechanisms contribute to treatment failure, commonly leading to the reactivation of the MAPK pathway. A variety of resistance mechanisms are enabled by the underlying heterogeneity and plasticity of melanoma cells. Moreover, it has become apparent that resistance to targeted therapy is underpinned by early functional adaptations involving the rewiring of cell states and metabolic pathways.

General significance: The evidence presented suggest that the use of a combinatorial treatment approach would delay the emergence of resistance and improve patient outcomes.

1. Background

Nearly half of cutaneous melanomas carry a genetic mutation that leads to a substitution in position 600 of the serine/threonine kinase BRAF [1]. BRAF is a key protein in the Mitogen-Activated Protein Kinase (MAPK) pathway, which regulates several important cellular functions, including proliferation, differentiation, migration and apoptosis. The demonstration of the mutant BRAF oncogene-addiction of melanomas led to the development of mutant specific BRAF inhibitors (BRAFIs), such as vemurafenib (Zelboraf), dabrafenib (Tafinlar), and encorafenib (Braftovi) [2–4]. FDA approval and broad clinical use of vemurafenib and dabrafenib was astoundingly achieved within 10 years of the BRAF oncogene discovery. Further clinical benefits were gained from combining BRAFIs with MEK inhibitors (MEKIs) such as cobimetinib (Cotellic), trametinib (Mekinist), and binimetinib (Mektovi), significantly improving response rates and overall survival [5–7]. Interestingly, some serious adverse effects, including cutaneous squamous-cell carcinoma, were attenuated by the combination therapy. However, nearly all patients treated with single-agent BRAFIs, or in combination with MEKIs, eventually developed resistance and relapsed on therapy.

2. Scope of review

In this review, we discuss our current understanding of the mechanisms that mediate drug resistance in melanomas treated with targeted therapy. Tumour relapse is driven by a subpopulation of drug-tolerant cells that persist treatment and remain viable, while the rest of the population gets killed.

Drug resistance is a multifaceted phenomenon and a complex scenario involving genetic, epigenetic and metabolic changes within the tumour cells as well as in the tumour microenvironment. Here, we categorised the various mechanisms underlying treatment failure into those conferring intrinsic, adaptive and acquired resistance (Fig. 1), and highlight potential strategies to overcome these mechanisms of resistance.

3. Intrinsic resistance

Around 1 in 5 melanoma patients treated with BRAFIs show disease progression on their first assessment during treatment, despite carrying a BRAFV600E mutation, indicating the presence of intrinsic resistance in a substantial proportion of cells within these tumours rendering drug resistance [8]. Pre-existing genetic alterations and endogenously secreted factors from stromal or tumour cells have been identified as
drivers of intrinsic resistance to BRAF inhibition in some BRAF V600E mutant melanoma cell lines and tumours.

3.1. PTEN loss

Phosphatase and tensin homolog (PTEN) is a tumour suppressor gene, and a major regulator of the phosphatidylinositol-3-kinase (PI3K) pathway [9]. Endogenous PTEN mutations and deletions are frequently identified in BRAF-mutant melanomas [10]. In vitro studies demonstrated that PTEN loss activates MAPK and PI3K/AKT pathways in BRAF-mutant melanomas, and confer intrinsic resistance by suppressing the BIM-mediated apoptosis [11]. Moreover, melanoma patients with a PTEN loss of function alterations/mutations treated with BRAFi monotherapy or BRAFi plus MEKi were found to have shorter median progression-free survival (PFS) and overall survival [9,12], but interestingly PTEN loss did not affect overall response rates. In fact, only 10% of PTEN-null BRAF mutant melanomas exhibit intrinsic resistance to BRAFis [9,13]. The above suggests that PTEN loss contribution to resistance to BRAFis in melanoma may be contextual, but overall can reduce treatment effectiveness.

3.2. Amplifications of cyclin D1

In BRAF-mutant melanomas, the activation of MAPK/PI3K pathways drives uncontrolled cell proliferation via dysregulation of the RB pathway (p16INK4A; cyclin D-CDK4/6; RB) [14]. Loss of p16 in melanoma cells can occur via deletions (50–80%), inactivating mutations (16%) and epigenetic silencing (20%) of CDKN2A [14]. In addition, the CCND1 gene, encoding for cyclin D1, is amplified in 11% of melanomas, including 17% of BRAF V600E melanomas [15]. Overexpression of cyclin D1 was linked to resistance to BRAFis in an earlier in vitro study [15]. In line with this observation, a clinical study found CDKN2A deletion and CCND1 amplification to be associated with shorted PFS in patients treated with dabrafenib [12].

3.3. RAC1 mutations

RAC1 is a member of the Rho family of small GTPase that regulates cell proliferation, cytoskeletal reorganization and cell migration [16]. The RAC1 P29S is the third most common hot-spot mutation (4%) in melanoma after BRAF V600E (50%) and NRAS Q61 (20%) [16,17]. The RAC1 P29S is an activating mutation leading to perpetual RAC1 phosphorylation resulting in increased cell proliferation and migration [16]. Endogenous RAC1 P29S in tumours correlated with early resistance and lack of response to BRAF inhibition [13]. Silencing the RAC1 P29S in BRAF V600E-mutant melanomas restored sensitivity to BRAFis, indicating the RAC1 P29S could be a predictive biomarker to RAF resistance in melanoma patients [13,18].

3.4. HOXD8 mutations

HOXD8 is a homeobox transcription factor that plays a crucial role in cell division, adhesion, proliferation, apoptosis and differentiation [19]. Dysregulated HOX expression has been reported in multiple malignancies [19]. A HOXD8 mutation was observed in the tumour from one patient in a comprehensive whole-exome sequencing study of 45 patients with early resistance to BRAFis [13]. In addition, an in vitro RNAi screening implicated HOXD8 suppression in resistance to a broad RAF inhibitor [20]. However, the significance of HOXD8 mutations for BRAFis remain unclear, as no other case has been reported to date.

3.5. MEK mutations

MEK1/2 proteins are downstream components of RAF protein that promote ERK phosphorylation and MAPK signalling [21]. Pre-existing MEK1 mutations are present in 5% of the melanomas [10,17]. Pre-existing MEK1 mutations have been attributed to shorter PFS in BRAF-mutant melanomas treated with BRAFis [22]. In vitro studies identified the MEK1 mutation, MEK1 Y121, confer resistance to BRAFis and MEKis [21].
3.6. NF1 loss

Neurofibromin 1 (NF1) was discovered to play an important role in the inhibition and suppression of RAS, constraining the MAPK pathway [20]. Genome scale screening using RNAi and CRISPR-Cas9 have implicated NF1 loss in the resistance to BRAFi and NF1 mutations were observed in BRAF-mutant tumour cells intrinsically resistant to RAF inhibition [20,23]. Importantly, endogenous NF1 alterations were found in pre-treatment tumours of patients who were refractory to vemurafenib treatment demonstrating the clinical significance of NF1-driven resistance [20].

3.7. Activation of c-Jun/RHOB axis

Ras homolog gene family, member B (RHOB) GTPase expression are induced by treatment of BRAF-mutant cell lines with BRAFis, via transcription factor c-Jun [24]. Low basal RHOB expression in melanoma cells lines correlated with BRAFi sensitivity, while depletion of RHOB restored sensitivity to MAPK inhibition [24]. Analysis of biopsies from patients treated with vemurafenib, indicated significantly shorter PFS in patients whose tumour samples displayed a positive RHOB staining before treatment compared to those with negative RHOB staining. It is thought that activation of the c-Jun/RHOB axis affects response to BRAFis through the activation of AKT pathway [24].

3.8. Factors secreted by tumour microenvironment

3.8.1. Hepatocyte growth factor /c-MET

Increased hepatocyte growth factor (HGF)/c-MET signalling mediates melanoma cell proliferation, invasion and offer protection from apoptosis [25]. In vitro studies demonstrated increased cell proliferation driven by the stromal HGF/c-MET signalling resulting in the reactivation of the MAPK and PI3K/AKT pathways, thereby rescuing melanoma cell lines from RAF and MEK inhibition [25,26]. Treatment with BRAFis plus HGF/c-MET inhibitors restored the sensitivity of these cells to BRAF inhibition, supporting the role of HGF signalling in melanoma resistance [25]. A recent study indicated that MAPK inhibition induced rapid increase in MET and GAB1 levels, priming the tumour cells for HGF-mediated rescue [27]. In contrast with previous studies implicating stromal cells in HGF secretion [25], tumour derived HGF was reported to convey resistance to BRAFis [27]. Nevertheless, treatment with a selective MET inhibitor completely attenuated HGF-mediated rescue, underscoring the role of the HGF/c-MET axis in mediating cell survival and resistance.

3.8.2. Hypoxia inducible factor

Hypoxia inducible factor 1α (HIF-1α) is a component of the HIF transcriptional factor which acts as an oxygen sensing machinery and a key modulator of the transcriptional responses under hypoxic stress and excess reactive oxygen species [28]. Under hypoxic conditions, melanoma cells exhibited decreased sensitivity to vemurafenib, trending towards resistance [28]. Proliferative melanoma cell lines exposed to hypoxic conditions transitioned into an invasive phenotype through a HIF-1α dependent transcriptional mechanism, indicating the role of HIF-1α in phenotype switching [29,30]. HIF-1α induced transcriptional programming has a profound effect on the central carbon metabolism, increasing glycolytic rates and decreasing mitochondrial respiration [31].

4. Adaptive response and drug tolerance

Despite the initial tumour reduction observed in most melanoma patients treated with BRAFis, complete tumour regression occurs rarely. This is due to the emergence of BRAFi induced compensatory
mechanisms, referred here as adaptive responses, that enhance the pro-survival and pro-proliferative capacity of a proportion of the original tumour population. These adaptive responses are temporary responses that are reversible, and non-transferable to progenies. Some of the adaptive mechanisms identified to date include, phenotypic switching or cell-state transitions, secretion of factors by the tumour microenvironment, and the more recently trending metabolic reprogramming.

4.1. Loss of negative feedback loops

BRAFi sensitive BRAFV600E mutant cells exhibit low expression of RAS-GTP before treatment, due to ERK-dependent feedback suppression of the receptor tyrosine kinases (RTK) signalling [32]. Active ERK can directly regulate signalling intermediates, such as EGFR and SOS, or indirectly activate the expression of negative feedback regulators such as SPROUTY (SPRY) and DUSP proteins [32] (Fig. 2). Inhibition of the MAPK pathway by BRAFis relieves this feedback, resulting in the re-activation of multiple pathways and attenuation of the antitumor effects of the targeted inhibitors [33]. This adaptation occurs within hours, thus diminishing the effectiveness of the therapy.

From the perspective of BRAFi resistance, it is important to note that the loss of these kinase-dependent negative feedback loops is unlikely to solely drive resistance, but they rather facilitate a subpopulation of tumour cells to survive in an adapted drug-tolerant state.

4.2. Cell-state transitions

The identification of distinctively high and low microphthalmia-associated transcription factor (MITF) levels within a melanoma tumour population [34] marked the conceptualization of the MITF-rheostat model [35]. MITF is a melanocytic-lineage transcriptional factor crucial for early melanogenesis and differentiation in melanocytes, and identified a master regulator of several biological processes in melanoma cells such as invasion, survival, cell cycle regulation and autophagy [30,36,37].

The MITFhigh population expressed increased levels of MITF downstream targets such as MLANA, PMEL, TYRP-1 and TYRP-2 genes, and are more proliferative and retained sensitivity to BRAFis [34,38,39]. On the other hand, the MITFlow population expressed genes associated with invasiveness such as high WNT ligand WNT5A, receptor tyrosine kinase AXL, TGFβ, TNFα/NF-κB activation, JUN and TEAD, and conferred resistance to targeted therapy [35,38,39]. Numerous studies confirmed the intrinsic resistance conferred by the AXLhigh and MITFhigh phenotype in response to MAPK inhibition in melanoma cells [38-41]. Activation of markers of invasiveness in the sensitive population reduced the MITF expression, with cells transitioning into a resistant phenotype.

In notable contrast with the above observations, upregulation of MITF has been identified as a driver of drug tolerance state and suppression of MITF pharmacologically sensitised the cells to MAPK inhibitors (MAPKi) [42]. High expression of MITF and its target genes (MLANA, PMEL, TYRP-1 and TYRP-2) with BRAFi treatment in BRAF-mutant melanomas was linked to resistance [43]. Upregulation in MITF expression as an early driver of non-mutational drug tolerant state in melanoma cells and linked to intrinsic resistance [13,39,42,44-48], and PAX3, an upstream regulator of MITF, was identified as a regulator of MITF expression [42]. Thus, both MITFhigh and MITFlow phenotypes have been linked to innate resistance in melanoma cells (Fig. 3).

Multiple studies have reported in a phenotypic switch between the “proliferative” and the “invasive” state in melanoma cells upon BRAFi treatment and associated with resistance [34,35,49-51]. Interestingly, such a phenotypic switch was activated by factors secreted by the tumour microenvironment and stress factors contributing to tumour plasticity [40,52]. Bulk sequencing and, more recently, single-cell RNA sequencing of melanoma tumours have confirmed the presence of these different states in individual cell within tumours [51,53]. This underlying tumour heterogeneity allows for rapid adaptation and survival of tumour cells early during treatment leading the emergence of resistance.

4.3. Metabolic reprogramming

Metabolic reprogramming is a hallmark of cancer, driven by oncogenic signalling pathways or poorly vascularized tumour microenvironment to meet the increasing cellular biomass needs [54]. BRAF-mutant melanoma cells exhibit high glycolytic activity and decreased mitochondrial respiration, to meet the increasing biomass and ATP needs of high proliferative cells [55]. The Warburg phenotype in melanomas is partially driven by the MAPK or P13K pathway by increasing the production of HIF-1α and MYC and promoting glycolysis or inhibiting MITF, a key regulator and promoter of oxidative phosphorylation (OXPHOS) in tumour cells [31]. Treatment with BRAFis and MEKis triggers metabolic programming in the BRAF-mutant cells to reduce glycolysis and increase mitochondrial respiration by activating MITF-PGC1α-OXPHOS pathway. Evidence of increased peroxisome proliferator-activated receptor γ coactivator 1α (PGC1α) levels, a marker of elevated OXPHOS, was exhibited by melanoma patients treated with single-agent BRAFi and in combination with MEKi [44,45]. KDM5B (JARID1B) expression has been indicated as a marker of these slow-cycling BRAFi resistant cells with increased oxidative phosphorylation [56]. Endogenous JARID1B and PGC1α overexpressing cells in patient tumours demark a subset of cells with increased mitochondrial capacity and resistance to oxidative stress that survive BRAFi [57,58].

In addition to the alteration in the glycolysis pathway, MAPK inhibition alters the fatty acid oxidation (FAO) in BRAF-mutant melanoma cells, which supply the structural material for cell and organelle membranes [59]. Short-term treatment with MAPK inhibition led to upregulation in fatty acid transporter CD36 in BRAF-mutant melanoma cells and PPARα-mediated and carnitine palmitoyl transferase 1A (CPT1A)-dependent FAO [59]. In parallel, fatty acid synthase, a key enzyme in endogenous fatty acid synthesis, is indirectly activated by the MAPK and P13K pathways [60]. Thus, the continuous signal for cell proliferation is supported by multiple metabolic pathways.

4.4. ER stress and autophagy

The nuclear translocation and reactivation of ERK drive a non-canonical ER stress response via ATF4 phosphorylation, to induce cytoprotective autophagy. Dephosphorylation of ERK activates the translocation of MAPK components from the cytoplasm to the ER by GRP78 (molecular chaperone), KSR2 (scaffolding protein), early endosomes and SEC6 (ER translocase) [61,62]. This translocation is required for the re-phosphorylation of ERK in the cytoplasm, which is carried out by the cytoplasmic lipid kinase domain of pERK [62].

Autophagy can be promoted by the activation of the MAPK pathway or by the LBK1-AMPK pathway to rescue the cells from glucose starvation [65]. BRAF inhibition also induces autophagy by the activation of the transcriptional factor, TFEB [66]. The “BRAF-TFEB-autophagy-lysosome” axis constitutes an intrinsic regulatory pathway in BRAF-mutant melanoma, coupling BRAF signalling with TGF-β signaling to drive tumour progression and chemoresistance [66].

5. Acquired resistance

Around 50% of patients treated with BRAFis alone or in combination with MEKis experience an initial significant shrinking of the tumour followed by tumour outgrowth, due to the emergence of acquire resistance. This resistance often spawns from the acquisition of a mutation that either reactivates the MAPK pathway or circumventing the MAPK pathway altogether through the utilisation of alternative pathways to support cellular growth.
5.1. MAPK reactivation-based survival

5.1.1. Receptor Tyrosine Kinases

Receptor tyrosine kinases (RTKs) act as upstream activators of MAPK signalling. Nazarian et al. first demonstrated that increased expression of PDGFRβ conferred resistance to BRAFis [67], which was further demonstrated by others using different cell lines [68,69]. In contrast, another study showed increased expression of EGFR, KIT and MET with decreased expression of PDGFRβ in resistant M249 cells [70]. Supra-physiologic levels of c-MET transcripts have been found BRAFi resistant melanomas [71]. A study by Shaffer et al. suggested that multiple RTKs, such as AXL, EGFR, PDGFRβ and JUN are expressed in small subpopulation of melanoma cells prior treatment by non-heritable, transient expression [41]. BRAFi treatment selects for an increased proportion cells expressing these RTKs, which mediate resistance through the activation of the MAPK pathway or alternative PI3K/AKT pathway.

5.1.2. NRAS and MEK mutations

NRAS serves as an activating mutation within melanoma encompassing around 28% of melanomas, with Q61R being the most common [10]. NRAS mutants preside within both combinational and mono-therapy cohorts [67,72]. NRAS mutations as a resistance mechanism have an occurrence of 5–18% [73–75]. Resistant cells with secondary NRASQ61K mutation require CRAF expression and SHOC2 scaffold protein to re-activate MAPK [76]. BRAF inhibition specifically, not drug binding, drives wild-type BRAF binding to CRAF and activation of MEK [77]. Despite the theoretical and preclinical support for CRAF overexpression to mediate BRAFi resistance it has yet to be reported within clinical samples [78].

MEK1 mutations are rare in melanoma and are often associated with either BRAF or NRAS mutations [10]. MEK1 mutations within either exon 3 or 6 were found to confer resistance to BRAF inhibition [79]. Further studies also supported that MEK1 mutations in BRAFV600E melanomas are linked to both intrinsic and acquired resistance to BRAFis [22,80]. Various meta-analysis studies have described an
overall incidence of 7–8% for MEK1/2 mutations in BRAFi mono-
therapy and BRAFi plus MEKi-resistant melanomas [72,73]. In con-
trast, Shi et al. showed that pre-existing exon 3 mutations, MEKPi
and MEKII, do not confer resistance to vemurafenib [81]. It has
been postulated MEK1 exon 3 mutations are not constitutively acti-
vating but render MEK1 more readily activated. Further studies are
required to disentangle the role of MEK mutations in pathogenesis and
treatment resistance of melanoma.

5.1.3. BRAF amplification
The overproduction of BRAFV600E due to the genetic amplification
of the mutant gene has been established as a common mechanism of
resistance to both BRAFi or BRAFi plus MEKi [72,82], confirmed by
various large studies of clinical specimens [72–75]. BRAFV600E ampli-
dication drives resistance through the excess generation of activated
MEK, which in turn activates downstream constituents of the MAPK
pathway. BRAF amplification and alternative splicing were observed
most frequently followed by NRAS mutations and MEK1/2 mutations
[73].

5.1.4. BRAF splicing variants
Resistance to the BRAFis can also be conferred through the pro-
duction of aberrantly spliced BRAFV600E isoforms that lack the RAS
binding domain (RBD) encoded by exons 3–5 [83]. These splicing
variants lacking the RBD, can dimerize in the presence of low levels of
RAS and confer drug resistance [83]. Four BRAF splicing variants have
been described, referred as p61, p55, p48 and p41 based on their pre-
dicted molecular weight. Alternative BRAF spliced isoforms have been
identified in patients progressing on BRAFi alone and in combination
with MEKis and as in preclinical models [83–86]. In fact, expression of
aberrantly spliced BRAFV600E isoforms mediates resistance in 13–30%
of melanoma patients [73–75]. Although BRAF splicing variants are capable of conferring resistance to BRAFi, cell line studies have shown
that melanoma cells carrying splicing variant remained susceptible to
MEK inhibition [72]. Moreover, enhanced association between BRAF
splicing variants and their substrate, MEK, that is required for re-
sistance to BRAFis [87].

5.1.5. COT alterations
The Ser/Thr MAP kinase MAP3K8 (or COT) has the potential to
directly phosphorylate MEK to trigger downstream cascades.
Johannessen et al. reported that COT expression was associated with
acquired resistance to BRAFi in melanoma cell lines and tissue obtained
from relapsing patients following treatment with MEKis or RAF
inhibitors [88]. Over activation of MEK within the cell line A375 was
established to occur through COT signalling, also generating resistance
to the MEKis, selumetinib and CI-1040 [88].

5.1.6. STAG 2 and 3 alterations
Loss-of-function mutations in STAG2 and decreased expression of
STAG2 and STAG3 proteins in several tumour samples from patients
with acquired resistance to BRAFi and in BRAFi-resistant melanoma cell
lines [89]. Furthermore, STAG3 mutations were found 3/14 pre-treat-
ment samples of patients who developed resistance vemurafenib within
12 weeks of treatment and the post-relapse sample of another 6 cases
[89], suggesting STAG2 mutations to mediate intrinsic as well as ac-
cquired BRAFi resistance (Fig. 2). STAG2 knockdown let to decreased
dual-specificity phosphatase 6 (DUSP6). DUSP6 acts as a negative
regulator of ERK activation [90]. Thus, STAG2/3 alterations result in
ERK activation, by limiting dephosphorylation.

5.1.7. BOP1 downregulation
The ribosome biogenesis protein Block of Proliferation 1 (BOP1)
acts as a regulator of DUSP4 and DUSP6 [90,91]. Unlike STAG
knockdown, which saw a reduction in DUSP6 but not DUSP4, loss of
BOP1 generated a reduction in both, leading to an increase in MAPK
signalling [89,91] (Fig. 2). A small scale investigation into patient
samples both pre and post BRAFi alone or in combination with MEKi
revealed a reduced protein expression of BOP1 within 7 of the 11 cases
that relapsed [91]. The results of this study, though initial highlight
another escape mechanism that can be utilised by melanomas.

5.2. MAPK independent based survival

5.2.1. PI3K-AKT pathway
Another established pathway is the PI3K/AKT pathway [92]. The
induction of the PI3K-AKT by insulin could protect BRAFV600E cells
from vemurafenib [93]. Cross-talk between the PI3K and MAPK path-
ways has been established, with BRAFI resistant cell lines utilising AKT
to trigger ERK within MAPK for cell survival, circumventing both
BRAFis and MEKis [94]. AKT1 mutant based resistance to BRAFis has
been identified previously in progressive patient samples to mono-
therapy [74]. Recent work demonstrated that PI3K activity is capable of
promoting survival but not proliferation of cell lines when challenged
with BRAFis plus MEKis [95].

5.2.2. WNT5A/β-catenin pathway
Continuous BRAFi in BRAF-mutant cell lines results in elevated
WNTS4A transcripts. Furthermore, 7 out of 11 tumours from patients
who progressed in BRAFis presented increased WNTS4A expression
compared to pre-treatment samples [96]. In vitro studies demonstrated
that a loss of WNT5A reduced the viability of the cells in the presence of
BRAFis [96]. WNT5A overcomes BRAF inhibition through the increased
phosphorylation of AKT and activation of RYK and F2D7 receptors
supporting non-canonical WNT signalling [96].

6. Potential strategies to overcome resistance
As discussed above, intrinsic and acquired resistance primarily in-
volve the reactivation of the MAPK pathway. Concomitant inhibition of
the BRAF and MEK nodes to overcome the MAPK signalling from hy-
peractivation of MEK has shown increase PFS, but still emerge similar
resistance mechanisms [72]. Reactivation of ERK is central to most
acquired resistance mechanisms. Thus, next generation ERK inhibitors
have been suggested as a suitable target for effective MAPK inhibition,
with molecules such as LY3214996 (NCT02857270) and BVD-523
(NCT02465060) progressing into clinical trial [97]. However, the suc-
cess of this approach remains uncertain considering the role of feedback
loops such as DUSP/SPRY which could lead to reactivation of the MAPK
pathway [32].

To address the upregulation of RTK as mechanism of resistance,
combination of BRAF/MEK blockade with RTK inhibitors (e.g.,
LY3022855, lapatinib and foretinib) are currently in trials to prevent
the MAPK reactivation (NCT03101254, NCT03455764) [98]. Increased
RTK signalling was reported to activate SRC/FAK/STAT3 signalling
leading to invasive phenotype [99]. Thus, combination of BRAF/MEK
blockade with SRC, FAK or STAT3 inhibitors (SAB298, saracatinib)
have been suggested to target the BRAFi-resistant melanoma population
with high dedifferentiated state or invasive phenotype [99].

Combination of BRAF/MEK with PI3K inhibitors have been trialled
to block the activation of parallel signalling by the AKT/PI3K pathway,
but found associated with increased toxicities or unmet therapeutic
goals due to rapid clearance [100]. Alternatively, combination of mTOR
inhibitors with BRAFi was thought to be effective in tumours with
PI3K/AKT pathway activation, by inducing apoptosis. Preclinical evi-
dence also demonstrated that the combination of mTOR inhibitors with
MAPKi to desensitise the high MITF expressing cells with high OXPHOS
and mitochondrial activity [45]. Initial phase I trials showed positive
outcome with limited toxicity (NCT10596140) [101], but treatment
efficiency evaluation in larger cohort of molecularly matched patients
is still needed. Another approach trialled in overcoming intrinsic and
acquired resistance is the combination of MAPKi and

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apoptosis induction by targeting molecules such as BCL-2 family protein (NCT01989585), CDK4/6 (NCT02645149) [102], histone deacetylase (NCT02836548) and heat shock protein 90 (NCT02097225) [103].

Acknowledging the understanding that cell plasticity enables the survival of a dormant population of MAPK-inhibited melanoma cells, multiple approaches have been tested to overcome adaptive resistance. For example, the combination of BRAFi with hydroxychloroquine is able to re-sensitize resistant cells by inhibiting cytoprotective autophagy induced by the ER stress response (NCT03754179) [61]. Other strategies have involved the inhibition of PAX3 mediated MITF upregulation using the HIV protease inhibitor nelfinavir [42], or by targeting AXL with the antibody conjugate AXL-107-MMAE [104]. On the other hand, nicotinamide phosphoribosyl transferase inhibitors and the combination of FAO and glycolysis inhibitors, may avoid the early metabolic reprogramming induced by MAPKi that underscores adaptive resistance [59,105].

Despite the multiple alternatives being tested, the benefits of these approaches might not be apparent in a clinical setting when patients harbour multiple active resistance mechanisms [106]. Clinical and preclinical evidence has demonstrated diverse resistance mechanisms within the same tumour, underscoring the contribution of genomic heterogeneity to BRAFi treatment failure. Hence, early treatment in the adjuvant setting may be the best strategy to avoid the emergence of resistance. In line with this, the introduction of combination MAPKi therapy into stage III melanoma has had a significant impact in the prevention of relapse post intervention [107].

7. Major conclusions

Overall, acquired resistance to BRAF inhibition depends on oncogenic signalling through reactivation of MAPK or activation of alternative pathway such as the PI3K/AKT pathway. Resistance can be acquired by upregulation of receptor tyrosine kinases signalling, by directly affecting genes in these pathways, or by enhancing downstream signalling. However, the mechanisms underlying acquired drug resistance are hugely diverse, with evidence of high inter- and intra-tumour heterogeneity [75]. These resistance mechanisms are distinct to those observed following targeted therapy treatment in other cancer types. For example, no secondary “gatekeeper” threonine mutations in BRAF have been observed, which is a common resistance mechanism to other kinase inhibitors [108,109].

As reviewed above, multiple studies have identified numerous mechanisms of resistance in patients failing single-agent BRAFis or in combination with MEKis. Interestingly, the vast majority lead to the reactivation of the MAPK pathway underscoring its importance for melanoma cell maintenance [110]. In addition, to those events observed only in treated tumours (acquired resistance), several resistance effectors already exist in pre-treatment samples (intrinsic resistance), in some cases constituting a tumour cell subpopulation. However, cumulative they still failed to explain all the observed clinical relapse cases. Thus, further studies are needed to elucidate the complete landscape of resistance mechanisms.

The picture is less clear for cellular processes conferring adaptive resistance. Prior to relapse, targeted therapy induces cellular adaptations to survive treatment, and within these cells acquire mechanism of resistance develops. Now it is better understood the role of tumour plasticity and metabolic reprogramming in these adaptation processes [51,111–113]. This has led to new treatment paradigms suggesting the combination of oncogenic inhibitors with metabolic targets. This may provide effective control of tumour growth, by avoiding the survival of cell subpopulations from which acquired resistance may emerge.

Finally, the emergence of effective immunotherapies provides alternative treatments with the potential to deliver long term control of melanoma growth [114]. The identification of increased tumour immune infiltrate in BRAFi treated melanomas, combination with immunotherapies were thought to enhance tumour control (NCT02902042, NCT02858921). However, these combinations have significant associated toxicities [115]. Sequential administration of targeted and immunotherapy also has its limitations, with translational studies suggesting that an immuno-resistant phenotype emerges on progression after BRAF inhibition [116]. Moreover, innate and adaptive BRAFi resistance mechanisms overlap with that found in tumours failing immunotherapy such as MITF/AXL programming, WNT pathway activation and PTEN loss [117]. In this context, the rationale for treatment selection may need to be based on the phenotypic and molecular characteristics of the pre-treatment tumours, which requires a clear and comprehensive understanding of the mechanism mediating resistance.

8. General significance

Although treatment with BRAFis provides rapid response in most melanoma patients, at present the emergence of resistance remains unavoidable. Numerous studies have addressed the mechanisms underlying the rapid emergence of resistance, but only half of these cases can be explained by the known mediators. Preclinical studies support the mechanisms observed in patients, indicating that the development of resistance is more complex than a single mutation. Further studies are required to better understand BRAFi resistance and to aid the developing strategies that can retain long-term durable responses of combination therapy.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

L.P.T. is supported by the ECU Higher Degree by Research Scholarship. M.E.C. is supported by ECU SMHS Research Scholarship. E.S.G. is supported by a fellowship from the Cancer Council WA.

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